Phosphorus and Mineral Concentrations in Whole Grain and Milled Low Phytic Acid (*lpa*) 1-1 Rice

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Cereal Chem. 82(5):517–522

ABSTRACT

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate) is the most abundant form of phosphorus (P) in cereal grains and is important to grain nutritional quality. In mature rice (*Oryza sativa* L.) grains, the bulk of phytic acid P is found in the germ and aleurone layer, deposited primarily as a mixed K/Mg salt. Phosphorus components and minerals were measured in whole grain produced by either the rice (*Oryza sativa* L.) cv. Kaybonnet (the nonmutant control) or the low phytic acid 1-1 (*lpa*1-1) mutant, and in these grains when milled to different degrees (10, 12, 17, 20, 22, and 25%, w/w). Phytic acid P is reduced by 42–45% in *lpa*1-1 whole grain as compared with Kaybonnet, but these whole grains had similar levels of total P, Ca, Fe, K, Mg, Mn, and Zn. In both genotypes, the concentration of phytic acid P, total P, Ca, Fe, K, Mg, and Mn in the milled products was reduced by 60–90%, as compared with whole grain. However, a trend was observed for higher (25–40%) total P, K, and Mg concentrations in *lpa*1-1 milled products as compared with Kaybonnet milled products. The reduction in whole grain phytic acid P in rice *lpa*1-1 is accompanied by a 5- to 10-fold increase in grain inorganic P, and this increase was observed in both whole grain and milled products. Phytic acid P was also reduced by 45% in bran obtained from *lpa*1-1 grain, and this was accompanied by a 10-fold increase in inorganic P. Milling had no apparent effect on Zn concentration. Therefore, while the block in the accumulation of phytic acid in *lpa*1-1 seed has little effect on whole grain total P and mineral concentration, it greatly alters the chemistry of these seed constituents, and to a lesser but detectable extent, alters their distribution between germ, central endosperm, and aleurone. These studies suggest that development of a low phytate rice might improve the nutritional quality of whole grain, milled rice and the bran produced during milling.

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexa*kis*phosphate) is the most abundant form of phosphorus (P) in seeds, typically representing 65–80% of seed total P, and from one to several percent of seed dry weight (Lott et al 2000). It also represents the most abundant form of *myo*-inositol phosphates (Ins phosphates) in seeds (Raboy 2003). Seed-derived dietary phytic acid may have a positive role as an antioxidant and anticancer agent (Graf and Eaton 1993). However, humans and other nonruminant animals such as poultry, swine, and fish excrete most of the phytic acid they consume. In the context of livestock production, this mostly represents a P-management issue, both in terms of supplying adequate nutrient P for optimal livestock productivity and in managing the disposal of waste P. In the context of human nutrition, the primary concern is the impact of dietary phytic acid on mineral cation retention, particularly with reference to iron and zinc nutrition. Phytic acid is an effective chelator of minerals such as iron, calcium, and zinc (Cilliers and van Niekerk 1986). The fact that phytic acid is poorly digested by humans and is an effective chelator of minerals can have a negative impact on the retention and utilization of minerals. Chronic consumption of phytic acid by populations dependent on cereals and legumes, which are rich sources of phytic acid, can contribute to mineral deficiency (Erdman 1981). In several developing countries, the consumption of rice provides the majority of the calories consumed. In many of these countries, mineral deficiencies are common (Brown and Solomons 1991).

In mature rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) grains, the bulk of whole grain phytic acid and minerals are found in the germ (embryo and scutellum) and aleurone layer (O'Dell et al 1972). Therefore, the removal of the outer layers of the rice and wheat grain through milling should reduce both milled grain phytic acid and minerals. While brown rice contains many beneficial nutrients, the presence of phytic acid might reduce its value in terms of mineral nutritional health. Ogawa et al (1979) demonstrated that early in rice grain development P, K, and Mg are evenly distributed throughout the central endosperm, aleurone layer, and germ tissues. As grains approach maturity, progressively more of the total P, K, and Mg is concentrated in the germ and aleurone, most of which is deposited as a mixed phytin salt of K and Mg, packaged into discreet inclusions referred to as globoids (Ogawa et al 1979; Liu et al 2004). Therefore, the ability of seeds to localize phytic acid synthesis might be part of the mechanism by which minerals such as K and Mg are also concentrated in specific tissues.

Low-phytate variants of maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), rice, and wheat have been developed through the isolation of low phytic acid (*lpa*) mutants in each species (Larson et al 2000; Raboy et al 2000; Raboy 2001; Dorsch et al 2003; Guttieri et al 2004). The first rice low-phytate mutant was isolated (Larson et al 2000) by screening a population of mutations induced in the cultivar Kaybonnet by γ-irradiation. Inheritance analysis indicated that the reduction in whole grain phytic acid P, ≈45% as compared with the nonmutant Kaybonnet control, was due to the inheritance of a single-gene mutation, rice low phytic acid 1-1 (*lpa*1-1) (Larson et al 2000). Normal Kaybonnet whole grains contained ≈2.23 mg of phytic acid P/g, whereas *lpa*1-1 grains contained ≈1.37 mg of phytic acid P/g. Reduced phytic acid P in rice *lpa*1-1 seeds did not appear to be due to a reduction in seed total P (≈3.12 mg of total P/g in Kaybonnet compared with ≈3.55 mg of total P/g in *lpa*1-1). Instead, the reduction in phytic acid P was largely matched, in terms of P, by an increase in whole grain inorganic P, from 0.14 mg of inorganic P/g in Kaybonnet to 1.13 mg of inorganic P/g in *lpa*1-1.

Liu et al (2004) conducted the first study of the distribution and deposition of P and minerals in rice *lpa*1-1 grains. Whole grains of Kaybonnet and *lpa*1-1 were dissected into two fractions: embryo, consisting of the embryo and scutellum; rest-of-grain, consisting of the central, starchy endosperm, and aleurone layer. Analyses of the mineral concentrations in these two fractions found no large differences between Kaybonnet (normal or wild-type control) and *lpa*1-1 in the whole grain total amount or distribution total P, K, Mg, Ca, Fe, or Zn (Liu et al 2004). However, the experimental

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DOI: 10.1094/CC-82-0517

design used could not detect differences in distribution between central endosperm and aleurone layer because both were contained within the rest-of-grain fraction.

If rice cultivars with the low phytate trait are produced, knowledge concerning the impact, if any, of the *lpa* mutation on the concentration of P components and mineral cations in milling products is of practical importance. In research described here, rice grain produced by Kaybonnet and the *lpa*1-1 mutant (Rutger et al 2003) were milled to different degrees, and the concentration of P components and minerals were assayed in the milled product. Milling removes the outer portions of the rice grain, including both germ and aleurone, producing two types of milled products enriched either in the central, starchy endosperm (white rice) or in the germ and aleurone (bran). Therefore, analyses of P and minerals in such products should also provide a test of the hypothesis that the localization of phytic acid synthesis in the cereal grain has a functional role in P and mineral localization.

MATERIALS AND METHODS

The rice cv. Kaybonnet and the low phytic acid 1-1 (*lpa*1-1) mutant were grown at the Dale Bumpers National Rice Research Center, Stuttgart, AR, in each of three years (2000, 2001, and 2002). Kaybonnet is the cultivar or genetic background from which the *lpa*1-1 mutant was isolated and serves here as the normal, nonmutant, or wild-type control. Grains were dehulled in a sample sheller (THU 35AI, Satake Engineering, Tokyo, Japan). The brown rice was subjected to different degrees of milling using a McGill #2 mill (Grain Machinery Manufacturing, Miami, FL). In grains harvested in 2001, milling products were obtained where 0% (whole grain), 10, 12, 17, 20, 22, or 25% of tissue was removed. In grains harvested in 2000 and 2002, milling products were obtained where 0% (whole grain), 10, 15, or 20% was removed. In addition, in 2000 and 2002, bran fractions, defined here as representing that portion of the grain removed by 10% milling, were obtained. Each sample was ground (Cyclotech grinder, Foss North America, Eden Prairie, MN) equipped with a 0.5-mm screen, and moistures were determined using Approved Method 44-15A (AACC International 2000).

Total P, phytic acid P, and inorganic P of the whole grain and milled products were measured using methods described earlier (Raboy et al 2000; Dorsch et al 2003). Briefly, samples of mature grain or milled products were dried for 48 hr at 60°C. These were then milled to pass through a 20-mm screen and stored in a desiccator until analysis. Total P was determined after wet-ashing of aliquots of tissue (150 mg) and colorimetric assay of P in the digests (Chen et al 1956). Inorganic P was determined colorimetrically after extraction of tissue samples (0.5 g) in 12.5% (w/v) TCA and 25 m*M* $MgCl_2$. The ferric-precipitation method was used to determine phytate P (Dorsch et al 2003). Aliquots of tissue (0.5 g) were extracted in 0.4*M* HCl and 0.7*M* Na_2SO_4. Phytic acid P was then obtained as a ferric precipitate, wet-ashed and assayed for P as in the total P analysis. All P-containing fractions are expressed as their P (atomic weight 31) content to facilitate comparisons. Phytic acid P can be converted to units of phytic acid (MW 660) by multiplying by the conversion factor 3.548.

Mineral cation composition of whole grain and milled products were determined by the University of Idaho Analytical Sciences Laboratory, Holm Research Center in Moscow, ID. Flour samples (1 g) were digested with nitric acid, and mineral concentrations in the digests were determined using a inductively coupled plasma-optical emission spectrometer (Perkin-Elmer Optima 3200 ICP-OES, University of Idaho, Moscow, ID). Analysis of variance (ANOVA) was conducted to determine *F* values useful in testing the effect on P and mineral concentrations of genotype, milling degree, and the interaction of genotype and milling degree. The general linear model (GLM) procedure (SAS Institute, Cary, NC) was used.

RESULTS AND DISCUSSION

The first analysis of P components and mineral cations in milled products of *lpa*1-1 grain was conducted using materials produced in 2001 (Table I). The concentrations of total P, phytic acid P, and inorganic P in Kaybonnet and *lpa*1-1 whole grain (0% milling) were similar to that initially reported by Larson et al (2000). Similar to the results of Larson et al (2000), little or no difference in whole grain total P was observed between these two genotypes, while whole grain phytic acid P was reduced ≈43% in *lpa*1-1, as compared with Kaybonnet, and this reduction was accompanied by a similar increase in whole grain inorganic P. In both genotypes, removal of outer portions of the grain through milling produced products with total P concentrations reduced by ≈49–76%, and phytic acid P levels were reduced by 63–92%, as compared with whole grains (Table I). However, concentrations of total P in the milled products of *lpa*1-1 grain were 16–48% higher than those

TABLE I
Phosphorus Concentrations in Rice cv. Kaybonnet and Low Phytic Acid (*lpa*) 1-1 Grain When Milled to Different Degrees, Production Year 2001

Milling Degree	Genotype	Total Phosphorus (mg/g ± SD)	Phytic Acid Phosphorus (mg/g ± SD)	Inorganic Phosphorus (mg/g ± SD)
0% (whole grain)	Kaybonnet	3.66 ± 0.30	2.54 ± 0.43	0.14 ± 0.04
	*lpa*1-1	3.62 ± 0.44	1.45 ± 0.32	0.97 ± 0.11
10%	Kaybonnet	1.59 ± 0.12	0.80 ± 0.04	0.09 ± 0.07
	*lpa*1-1	1.85 ± 0.16	0.53 ± 0.01	0.51 ± 0.06
12%	Kaybonnet	1.47 ± 0.05	0.69 ± 0.02	0.11 ± 0.08
	*lpa*1-1	1.75 ± 0.30	0.43 ± 0.03	0.46 ± 0.07
15%	Kaybonnet	1.14 ± 0.18	0.49 ± 0.03	0.13 ± 0.17
	*lpa*1-1	1.69 ± 0.26	0.43 ± 0.02	0.46 ± 0.08
17%	Kaybonnet	1.12 ± 0.07	0.37 ± 0.02	0.06 ± 0.04
	*lpa*1-1	1.43 ± 0.22	0.25 ± 0.03	0.38 ± 0.10
20%	Kaybonnet	1.04 ± 0.03	0.33 ± 0.03	0.08 ± 0.06
	*lpa*1-1	1.35 ± 0.07	0.15 ± 0.05	0.40 ± 0.10
22%	Kaybonnet	1.02 ± 0.06	0.23 ± 0.02	0.10 ± 0.10
	*lpa*1-1	1.31 ± 0.07	0.11 ± 0.05	0.38 ± 0.09
25%	Kaybonnet	0.87 ± 0.06	0.21 ± 0.04	0.08 ± 0.08
	*lpa*1-1	1.20 ± 0.06	0.13 ± 0.05	0.38 ± 0.09
ANOVA *F* value				
Genotype (G)		26***	46***	231***
Milling faction (M)		115***	118***	9***
(G × M)		ns	9***	6***

observed in milled products of Kaybonnet. This increase in total P in milled *lpa*1-1 products appears to be largely due to the 4- to 6-fold increases in inorganic P (Table I).

The concentration of each of the seven minerals cations (Ca, Cu, Fe, K, Mg, Mn, and Zn) was similar in whole grain (0% milling) of Kaybonnet and *lpa*1-1 harvested in 2001 (Table II). This confirms the results of Liu et al (2004), indicating that the *lpa* 1-1 mutation has little effect on whole grain total concentrations of both P (Table I) and mineral cations. Milling had little detectable effect on Cu and Zn in both Kaybonnet and *lpa*1-1 grain. In the case of Fe, a trend for reduced concentration with milling (≈9–54%) was observed in 2001 grain of both genotypes but was not statistically significant in this experiment (Table II). In contrast, removal of tissues through milling of grain of both genotypes produced fractions with substantially reduced concentrations of Ca, K, Mg, and Mn, as compared with whole grain. These reductions had a range of ≈35–60% for Ca and K, 60–80% for Mn, to 70–90% for Mg (Table II). There was little effect of genotype on the Ca and Mn concentrations of the 2001 milled products. While there was a statistically significant effect of genotype on Mg concentration in the 2001 milled products (increases in Mg concentration in milled products obtained from *lpa*1-1 as compared with Kaybonnet) (Table II), this was due to modest increases of 15–30% and was observed only in a subset of milled products of *lpa*1-1. A more pronounced effect of genotype on K concentration was observed in the milled products of 2001 grains. Milled products of *lpa*1-1 grain had consistently higher concentrations of K (29–66%) as compared with Kaybonnet (Table II).

To provide a test of the trends observed in the analyses of grains produced in 2001, grains produced in 2000 and 2002 were subsequently milled and the products analyzed for P and minerals (Tables III and IV). In this follow-up analysis, bran fractions, defined here as those portions of the grains removed in the 10% milling, were also analyzed. With one important exception, the results of analyses of P components were similar in the 1st round (2001) and 2nd round (2000, 2002). Kaybonnet and *lpa*1-1 whole grain total P concentration were similar (Table III). The level of phytic acid P reduction in *lpa*1-1, and the accompanying increase in inorganic P, were largely similar to that observed in 2001 grain. The difference in results is that in both 2000 and 2002 grains, the trend for increased total P in the milled products of *lpa*1-1 was either too small to be detected as statistically significant with the methods used here (Year 2000), or if statistically significant (Year 2002), the increase in total P in *lpa*1-1 milled products, as compared with Kaybonnet, was more modest than that observed in Year 2001 grains.

The concentration of total P in the bran fractions obtained from grains of both genotypes harvested in Years 2000 and 2002, was ≈15- to 30-fold higher than concentrations in milled products (Table III). The 45% reduction in phytic acid P in bran obtained from *lpa*1-1 grain, as compared with Kaybonnet is, on a percentage basis, similar to that observed in whole grain of these genotypes. In Kaybonnet bran, phytic acid P represents ≈2% of bran dry weight, and if converted into units of phytic acid (by multiplying by the conversion factor 3.548), represents ≈6.5% of bran dry weight. Since K and Mg together represent ≈2.5% of bran dry weight (Table IV), a K/Mg phytate salt might represent 8–10% of the Kaybonnet bran dry weight, a significant fraction of total bran mass. In this context, the reduction in phytic acid P in *lpa*1-1 bran represents ≈1.0% of bran dry weight, or in units of phytic acid, ≈3.5% of bran dry weight. This reduction is accompanied by a 10- to 15-fold increase in bran inorganic P (Table III). However, the increase in inorganic P in *lpa*1-1 bran, representing ≈0.5% of bran dry weight, does not entirely account for the decrease in phytic acid P. Thus, the decrease in bran phytic acid P might be accompanied by increases in inorganic P and other unknown forms of P not measured here. Alternatively, this result might be an artifact of the methods used here; for example, a possible underestimation of inorganic P in *lpa*1-1 bran. Further studies will have to address this question.

The mineral analyses of whole grains produced in Years 2000 and 2002, and milled products (Table IV), also yielded results similar to those of the analyses of Year 2001 grain, with one notable exception. Milling clearly reduced the concentrations of Ca, K, Mg, and Mn in the milled products of both Kaybonnet and *lpa*1-1, and again had less of an effect on Cu, Fe, and Zn concentrations. Only in K and Mg were there any statistically significant differences between *lpa*1-1 and Kaybonnet. However, while the trend for increased Mg in *lpa*1-1 milled products as compared with Kaybonnet was observed in both Years 2000 and 2002, the trend for increased K was only observed in Year 2002. In milled products from Year 2000 grain, K concentration was higher in Kaybonnet milled grain.

The bran fractions obtained from 10% milling of *lpa*1-1 and Kaybonnet grain had, in nearly all cases, similar levels of all minerals studied (Table IV). The relatively modest differences in

TABLE II
Mineral Concentrations in Rice cv. Kaybonnet and Low Phytic Acid (*lpa*) 1-1 Grain When Milled to Different Degrees, Production Year 2001

Milling Degree	Genotype	Ca (μg/g ± SD) (n = 2)	Cu (μg/g ± SD) (n = 3)	Fe (μg/g ± SD) (n = 3)	K (mg/g ± SD) (n = 2)	Mg (μg/g ± SD) (n = 2)	Mn (μg/g ± SD) (n = 3)	Zn (μg/g ± SD) (n = 3)
0% (whole grain)	Kaybonnet	110 ± 0.0	5.7 ± 4.0	11 ± 1.0	3.0 ± 0.10	1,200 ± 00	47 ± 4	22 ± 0.6
	*lpa*1-1	115 ± 7.0	5.0 ± 2.6	13 ± 1.2	3.0 ± 0.07	1,250 ± 71	49 ± 9	22 ± 3.8
10%	Kaybonnet	68 ± 17.0	8.8 ± 5.7	9 ± 5.6	1.4 ± 0.0	340 ± 28	19 ±1	18 ± 1.5
	*lpa*1-1	50 ± 3.5	5.9 ± 3.4	9 ± 3.0	1.9 ± 0.07	410 ± 28	18 ±1	24 ± 5.5
12%	Kaybonnet	56 ± 0.7	4.0 ± 2.0	9 ± 2.1	1.4 ± 0.07	345 ± 7	19 ± 1	20 ± 2.0
	*lpa*1-1	47 ± 4.2	4.2 ± 0.6	9 ± 3.6	1.8 ± 0.07	360 ± 28	17 ± 1	21 ± 5.5
15%	Kaybonnet	49 ± 1.4	5.4 ± 4.2	7 ± 2.9	1.1 ± 0.0	225 ± 35	16 ± 0.6	18 ± 0.6
	*lpa*1-1	50 ± 2.8	5.7 ± 3.3	10 ± 3.0	1.7 ± 0.14	360 ± 28	17 ± 1	21 ± 0.6
17%	Kaybonnet	48 ± 3.5	4.7 ± 2.9	10 ± 2.3b	1.1 ± 0.07	190 ± 14	16 ± 1	20 ± 2.3
	*lpa*1-1	46 ± 0.7	6.0 ± 3.7	11 ± 4.4	1.5 ± 0.14	250 ± 14	15 ± 1	19 ± 1.2
20%	Kaybonnet	49 ± 0.7	11.9 ± 15.7	7 ± 4.2	1.1 ± 0.07	165 ± 21	16 ± 1	18 ± 2.5
	*lpa*1-1	35 ± 4.9	3.1 ± 1.8	6 ± 1.3b	1.6 ± 0.00	135 ± 35	12 ± 0.6	19 ± 0.6
22%	Kaybonnet	42 ± 0.0	2.5 ± 1.0	7 ± 0.8b	0.9 ± 0.02	130 ± 14	14 ± 0	19 ± 3.1
	*lpa*1-1	36 ± 0.7	2.8 ± 1.3	7 ± 1.1	1.5 ± 0.00	135 ± 7	12 ± 0.6	19 ± 1.2
25%	Kaybonnet	45 ± 2.1	2.3 ± 0.7	7 ± 0.6b	1.0 ± 0.07	106 ± 20	14 ± 0.6	20 ± 4.7
	*lpa*1-1	36 ± 2.8	5.8 ± 3.6	8 ± 0.4b	1.6 ± 0.07	135 ± 7	12 ± 1	19 ± 2.0
ANOVA F value								
Genotype (G)		13**	ns	ns	331***	18***	ns	ns
Milling fraction (M)		87***	ns	ns	264***	687***	115***	ns
(G × M)		ns	ns	ns	8***	3*	ns	ns

TABLE III
Phosphorus Concentrations in Rice cv. Kaybonnet and Low Phytic Acid (*lpa*) 1-1 Mutant Grain When Milled to Different Degrees, Production Years 2000 and 2002

Year and Milling Degree	Genotype	Total Phosphorus (mg/g ± SD)	Phytic Acid Phosphorus (mg/g ± SD)	Inorganic Phosphorus (mg/g ± SD)
2000				
0% (whole grain)	Kaybonnet	3.50 ± 0.07	2.33 ± 0.11	0.12 ± 0.01
	*lpa*1-1	3.24 ± 0.32	1.28 ± 0.05	0.86 ± 0.01
10%	Kaybonnet	1.34 ± 0.05	0.60 ± 0.01	0.07 ± 0.01
	*lpa*1-1	1.43 ± 0.10	0.30 ± 0.01	0.30 ± 0.03
15%	Kaybonnet	0.99 ± 0.01	0.24 ± 0.01	0.07 ± 0.01
	*lpa*1-1	1.08 ± 0.04	0.20 ± 0.01	0.16 ± 0.01
20%	Kaybonnet	0.91 ± 0.02	0.18 ± 0.02	0.07 ± 0.01
	*lpa*1-1	1.10 ± 0.03	0.13 ± 0.01	0.14 ± 0.01
Bran	Kaybonnet	27.27b	18.44 ± 1.03	0.51 ± 0.03
	*lpa*1-1	26.85b	10.10 ± 0.45	5.34 ± 0.72
ANOVA *F* Value				
Genotype (G)		ns	289***	3,068***
Milling fraction (M)		ns	1,331***	1,272***
(G × M)		ns	123***	961***
2002				
0% (Whole Grain)	Kaybonnet	3.11 ± 0.02	2.36 ± 0.14	0.07 ± 0.01
	*lpa*1-1	3.47 ± 0.03	1.37 ± 0.01	0.92 ± 0.03
10%	Kaybonnet	1.15 ± 0.03	0.55 ± 0.03	0.04 ± 0.01
	*lpa*1-1	1.50 ± 0.05	0.40 ± 0.04	0.38 ± 0.03
15%	Kaybonnet	0.89 ± 0.04	0.22 ± 0.01	0.03 ± 0.01
	*lpa*1-1	1.29 ± 0.03	0.21 ± 0.01	0.27 ± 0.01
20%	Kaybonnet	0.88 ± 0.05	0.15 ± 0.01	0.03 ± 0.01
	*lpa*1-1	1.09 ± 0.02	0.14 ± 0.01	0.23 ± 0.01
Bran	Kaybonnet	27.32b	19.28 ± 1.99	0.43 ± 0.06
	*lpa*1-1	22.80b	10.42 ± 0.89	6.14 ± 0.03
ANOVA *F* Value				
Genotype (G)		336***	122***	3,660***
Milling fraction (M)		3,645***	922***	623***
(G × M)		5*	79***	491***

TABLE IV
Mineral Concentrations in Rice cv. Kaybonnet and Low Phytic Acid (*lpa*) 1-1 Grain When Milled to Different Degrees, Production Years 2000 and 2002

Year and Milling Degree	Genotype	Ca (µg/g ± SD)	Cu (µg/g ± SD)	Fe (µg/g ± SD)	K (mg/g ± SD)	Mg (µg/g ± SD)	Mn (µg/g ± SD)	Zn (µg/g ± SD)
2000								
0% (Whole Grain)	Kaybonnet	110 ± 10	1.8 ± 0.2	12 ± 1.5	3.07 ± 0.21	1,233 ± 58	48 ± 4.0	22 ± 3.6
	*lpa*1-1	103 ± 5.8	1.6 ± 0.4	16 ± 3.0	3.07 ± 0.15	1,267 ± 58	41 ± 2.1	25 ± 5.3
10%	Kaybonnet	51 ± 3.6	1.4 ± 0.2	BDL	1.60 ± 0.10	299 ± 42	17 ± 1.5	20 ± 2.1
	*lpa*1-1	55 ± 6.7	1.3 ± 0.3	5 ± 1.0	1.37 ± 0.15	340 ± 66	16 ± 1.5	24 ± 3.6
15%	Kaybonnet	43 ± 2.1	1.5 ± 0.4	BDL	1.30 ± 0.10	130 ± 26	14 ± 1.5	21 ± 7.6
	*lpa*1-1	44 ± 3.6	1.2 ± 0.2	BDL	1.03 ± 0.06	183 ± 21	13 ± 0.6	21 ± 1.2
20%	Kaybonnet	39 ± 2.9	1.3 ± 0.1	6 ± 1.0	1.20 ± 0.00	81 ± 7	14 ± 2.1	19 ± 2.9
	*lpa*1-1	40 ± 1.5	1.3 ± 0.5	BDL	0.96 ± 0.04	133 ± 8	12 ± 0.6	20 ± 0.6
Bran	Kaybonnet	595 ± 64	4.0 ± 0.4	93 ± 66	16.00c	8,700c	315 ± 7.1	72c
	*lpa*1-1	570 ± 42	5.4 ± 0.4	43 ± 2.1	17.50 ± 2.12	8,550 ± 919	255 ± 21	77 ± 3.5
ANOVA *F* value								
Genotype (G)		ns	ns	ns	14**	7*	12**	ns
Milling fraction (M)		231***	ns	25***	361***	984***	346***	ns
(G × M)		ns	ns	ns	ns	ns	ns	ns
2002								
0% (whole grain)	Kaybonnet	101 ± 8.5	1.4 ± 0.3	12 ± 1.5	2.80 ± 0.17	1,581 ± 33	43 ± 4.2	23 ± 4.4
	*lpa*1-1	101 ± 8.1	1.7 ± 0.2	13 ± 1.5	2.83 ± 0.25	1,233 ± 58	50 ± 4.0	25 ± 1.5
10%	Kaybonnet	45 ± 3.6	1.0 ± 0.2	8 ± 2.6	1.10 ± 0.10	230 ± 46	15 ± 1.5	18 ± 1.5
	*lpa*1-1	56 ± 9.6	1.4 ± 0.2	BDL	1.53 ± 0.05	363 ± 15	17 ±1.0	23 ± 4.7
15%	Kaybonnet	42 ± 3.8	1.0 ± 0.1	8 ± 1.6	0.91 ± 0.04	119 ± 22	12 ± 0.6	19 ± 1.0
	*lpa*1-1	39 ± 0.6	1.2 ± 0.3	BDL	1.23 ± 0.06	177 ± 6	13 ± 0.6	21 ± 4.4
20%	Kaybonnet	39 ± 2.5	0.9 ± 0.1	BDL	0.85 ± 0.04	82 ± 8.5	12 ± 0.6	18 ± 0.6
	*lpa*1-1	36 ± 3.5	1.2 ± 0.0	5 ± 0.8	1.17 ± 0.06	129 ± 28	12 ±1.0	19 ± 1.0
Bran	Kaybonnet	620 ± 14.1	5.4 ± 0.1	51 ± 4.9	17.00 ± 0.00	8,750 ± 212	305 ± 7.0	83 ± 1.4
	*lpa*1-1	610 ± 0.0	6.3 ± 0.0	47 ± 4.2	15.00± 0.00	8,900 ± 141	350 ± 0.0	71 ± 1.4
ANOVA *F* value								
Genotype (G)		ns	15**	ns	31***	33***	7*	ns
Milling fraction (M)		154***	8**	16**	300***	1,189***	341***	4*
(G × M)		ns	ns	ns	ns	ns	ns	ns

mineral concentrations between *lpa*1-1 and Kaybonnet milled products, such as those observed in K and Mg concentration, are small in absolute terms when compared with the concentrations of these minerals in the bran (Table IV). Therefore, while the *lpa*1-1 does appear to alter the distribution of P, K, and Mg in milled products, this effect is not large enough to produce noticeable differences in bran mineral concentrations, at least with the methods used here.

An *lpa* mutant called JS-12-LPA has been isolated in wheat (Guttieri et al 2004). In terms of whole grain P fractions and phytic acid P reduction, it is very similar to rice *lpa*1-1. In JS-12-LPA, whole grain phytic acid P is reduced by ≈37% as compared with the normal control JS-12-WT, but total P is unchanged. The reduction in phytic acid P is largely matched by an increase in inorganic P. The distribution of P and minerals in this low-phytate wheat mutant was studied with an approach similar to that used here, but different to the approach of the Liu et al (2004) rice *lpa*1-1 study. In the wheat study, whole grain produced by JS-12-WT and JS-12-LPA were subjected to milling, producing break flour (obtained after the first pass through the mill rolls), reduction flour (obtained after a second pass through the mill rolls), and bran (the grain portion removed during milling). The milled flours are enriched with the central, starchy endosperm, whereas the bran is enriched with the germ and aleurone layer. Essentially no differences between JS-12-WT and JS-12-LPA were observed in the concentration of total P, Ca, Fe, K, Mg, Mn, and Zn between these different milling fractions. The only statistically significant difference attributable to genotype was a higher concentration of Cu in bran and other fractions of JS-12-WT as compared with JS-12-LPA. No effect of the rice *lpa*1-1 on Cu was observed here. In addition, Liu et al (2004) demonstrated that the rice *lpa*1-1 mutation does not alter the distribution of P and minerals between germ and the rest-of-grain, a fraction containing both aleurone layer and central endosperm.

In contrast, the results reported here demonstrate that, in addition to altering the chemistry of whole grain P, the rice *lpa*1-1 mutation also tends to increase the amount of P, K, and Mg in the milled products (*lpa*1-1 white rice), fractions that are enriched in central, starchy endosperm. This result does indicate that the localization of phytic acid synthesis and deposition is important to the distribution of P, K, and Mg in the mature grain. These increases were not very large, especially in light of the concentration of these minerals in the bran fractions. The *lpa*1-1 mutation also greatly alters the amount of phytic acid P and inorganic P in bran produced by milling. When chickens were fed a diet where rice bran, wheat bran, corn bran, soy bran, and oat hulls were used as fiber, only the ones fed rice bran had reduced body growth (Juliano 1994). This was attributed to higher phytic acid level in the rice bran diet (1.3%) as compared with that in other diets (0–0.4%). These findings might, therefore, be important in efforts to improve the nutritional quality of both white rice and rice bran side products.

The findings reported here need to be tested in three types of additional experiments. First, the *lpa*1-1 mutation should be crossed into other rice germplasms or cultivars. Grains produced by lines converted to homozygosity for *lpa*1-1 would then be milled and analyzed as done here. This would test whether the increases in white rice mineral content due to the *lpa*1-1 mutation is a heritable trait transferable to other lines. Second, this type of analysis should be conducted on rices grown in several different environments and production conditions. This would also test the general nature of these of these findings. Finally, and perhaps most importantly, rice mutations that have larger effects on grain phytic acid P need to be isolated and studied. For example, in barley, *lpa* mutations that reduce phytic acid by 50–90% have been isolated (Dorsch et al 2003). A similar analysis of a rice *lpa* mutation that causes a larger reduction in grain phytic acid would provide a powerful test of the findings reported here. Such mutations might also have a greater impact on the distribution of P and minerals in milled rice products.

CONCLUSIONS

A low phytic acid mutant (*lpa*1-1) and its parental cultivar Kaybonnet were milled to different degrees, and the total P, phytic acid P, inorganic P, and mineral cation (Ca, Cu, Fe, K, Mg, Mn, and Zn) concentrations were determined. The phytic acid P concentration in the whole grain, milled products, and bran fractions of *lpa*1-1 was ≈45% lower than that of similar products of Kaybonnet. While this large change in whole and milled grain P chemistry was not associated with large changes in the concentration of total P concentration in these products, a trend for increased total P in the milled products of *lpa*1-1, as compared with Kaybonnet, was observed. Reduced phytic acid P was accompanied by nearly equivalent increases in inorganic P. While large effects on the concentrations of mineral cations were not observed, a trend for increased K and Mg in the milled products of *lpa*1-1, as compared with Kaybonnet, was observed. Thus, the nutritional value of white rice and rice bran for uses as human foods or in animal feeds might be enhanced by the use of mutations like *lpa*1-1. Such mutations decrease phytic acid, a compound that is considered an antinutrient in terms of mineral nutrition, and might also increase the levels of desirable nutrients like inorganic P, K, and Mg, in milled products such as white rice.

ACKNOWLEDGMENTS

We acknowledge Amanda Lewis and Deborah Rowbury at the USDA-Aberdeen location, and Gordon Miller and Jeannie Wilson in Arkansas, for their assistance in conducting experiments and collecting data.

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[Received October 22, 2003. Accepted May 2, 2005.]